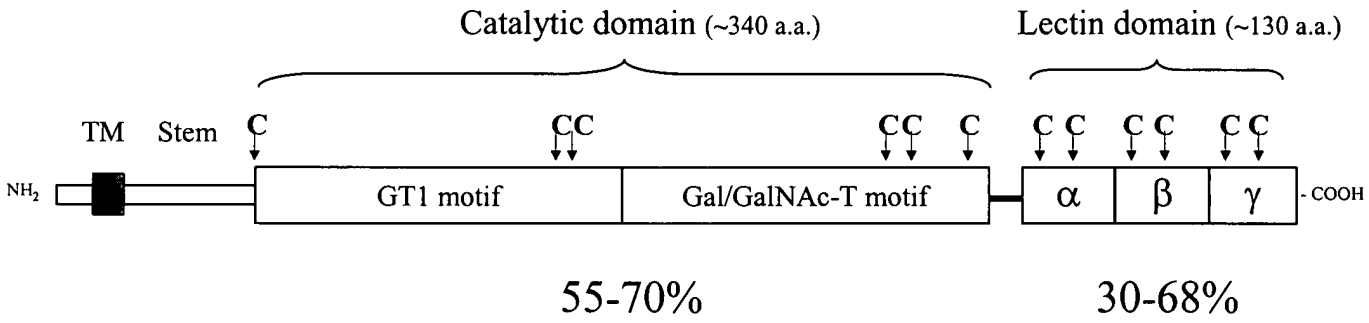
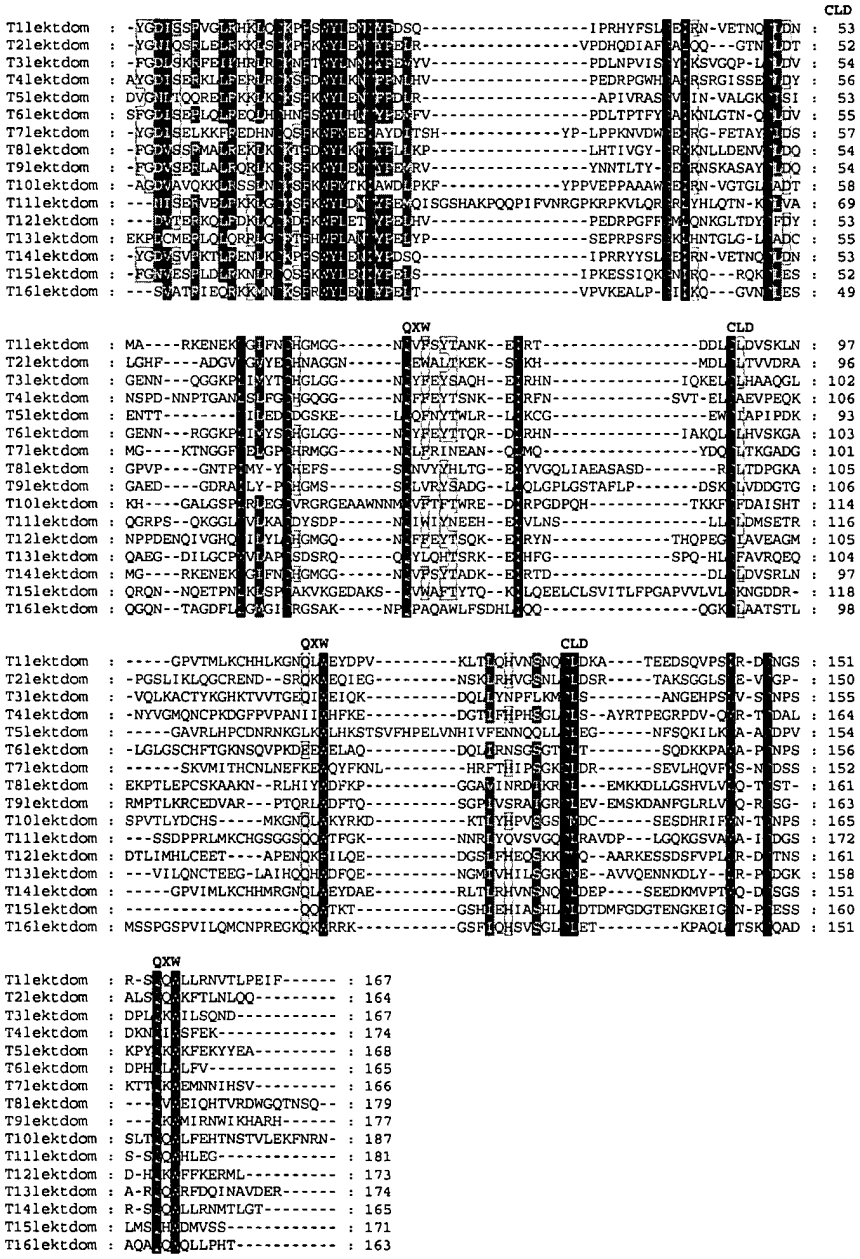

Catalytic domain (~340 a.a.)
Lectin domain (~130 a.a.)
TM
Stem
NH2
GT1 motif
Gal/GalNAc-T motif
α
β
γ
COOH
55-70%
30-68%

FIG. 1


CLD
T1lektdom : -YGDISSPVGLKHKLQCKPFSKYLENLMPDSQ----------IPRHYFSLKPRN-VETNQTDN : 53
T2lektdom : -YGHIQSPLLPLKKLSSKPFKKYLENTVPDLR----------VPDHQDIAFPALQQ---GTNTLDT : 52
T3lektdom : -FGDLSKKSPDIHHRLRTNFTYYLNNLVPDYV----------PDLNPVISYLKSVGQP-LTLDV : 54
T4lektdom : AYGDISPRKLLPERLRTSFSHDYILKNLPPNLHV----------PEDRPGWHLAARSRGISSPTDY : 56
T5lektdom : DVGNLTQQRPLPKKLKLSFKKYLENVPPDLR----------APIVRASYVLIN-VALGKNTSI : 53
T6lektdom : SFGDISPPLQLPEQLHHNFSKYLHNVPEFV----------PDLTPTFYLALKNLGTN-QTLDV : 55
T7lektdom : -YGDISELKKFPEDHNLQSFKKFYEEVAYDITSH----------YP-LPPKNVDWPERG-FETAYTDS : 57
T8lektdom : -FGDWSSPMALREKLKLKIFDDYILKNVPLLKP----------LHTIVGY-PRKNLLDENVTDQ : 54
T9lektdom : -FGDVSPRLALPQRLKLKFSKKYLENLVPEVRV----------YNNTLTY-PERNSKASAYTDQ : 54
T10lektdom : -AGDVAVQKKLRSSLNLSFKKFFYTKAAWDLPKF----------YPPVEPPAAAWPERN-VGTGLADT : 58
T11lektdom : ---NISPRVPLPKKLQLSFKKYLDNVPEWQISGSHAKPQQPIFVNRGPKRPKVLQRPLYHLQTN-KTVA : 69
T12lektdom : ---DVTPRKQLPDKLQLDFKKFLETVPELHV----------PEDRPGFFAWLQNKGLTDYTFDY : 53
T13lektdom : EKPPCMPPLQLQRPLGLTFTHKFLANVPPLYP----------SEPRPSFSPKKHNTGLG-LADC : 55
T14lektdom : -YGDVSVPKTLPENLKLKPFSKYLELVPDSQ----------IPRRYYSLKPRN-VETNQTDN : 53
T15lektdom : -FGNVESPLDLPKNLRLQSFKKYLEIVPELS----------IPKESSIQKPNIRQ---RQKTLES : 52
T16lektdom : ---SVATPIEQPKKLNLKSFRKYLEIVPELT----------VPVKEALP-PTLKQ---GVNTLES : 49

QXW
CLD
T1lektdom : MA----RKENEKLGLFNLHGMGG------NLVPSYTANK--ELRT----------DDLSLDVSKLN : 97
T2lektdom : LGHF----ADGVGGYYEGHNAGGN------LEWALTKEK--SLKH----------MDLSLTVVDRA : 96
T3lektdom : GENN---QGGKPLIYTGHGLGG------NLYFEYSAQH--ELRHN----------IQKELSLHAAQGL : 102
T4lektdom : NSPD-NNPTGANLSLFGHGQGG------NLFFEYTSNK--ELRFN----------SVT-ELSAEVPEQK : 106
T5lektdom : ENTT--------IIEDLDGSKE------LQQFNYTWLR--ILKCG----------EWSLAPIPDK : 93
T6lektdom : GENN---RGGKPLIYYSGHGLGG------NLYFEYTTQR--DLRHN----------IAKQLSLHVSKGA : 103
T7lektdom : MG----KTNGGFPELGPGHRMGG------NLLFRINEAN--QLMQ----------YDQSLTKGADG : 101
T8lektdom : GPVP----GNTPLMY-YGHEFS------SLNVYYHLTG--ELYVGQLIAEASASD--------RSLTDPGKA : 105
T9lektdom : GAED----GDRALLY-PGHGMS------SLVRYSADG--ILQLGPLGSTAFLP-------DSKSLVDDGTG : 106
T10lektdom : KH----GALGSPPRLEGSVRGRGEAAWNNMAVTFTWRE--DLRPGDPQH----------TKKFSFDAISHT : 114
T11lektdom : QGRPS--QKGGLAVLKADYSDP------NLIWIYNEEH--ELVLNS----------LLSLDMSETR : 116
T12lektdom : NPPDENQIVGHQLIYLGHGMGQ------NLFFEYTSQK--ELRYN----------THQPEGSLAVEAGM : 105
T13lektdom : QAEG--DILGCPVVLAPLSDSRQ------QLYLQHTSRK--ELHFG----------SPQ-HLSFAVRQEQ : 104
T14lektdom : MG----RKENEKLGLFNLHGMGG------NLVPSYTADK--ELRTD----------DLSLDVSRLN : 97
T15lektdom : QRQN--NQETPNLKLSPLAKVKGEDAKS--LVWAFTYTQ--KLLQEELCLSVITLFPGAPVVLVLKNGDDR- : 118
T16lektdom : QGQN--TAGDFLLGGGIIRGSAK-----NPLPAQAWLFSDHLLQQ----------QGKSLAATSTL : 98

QXW
CLD
T1lektdom : -----GPVTMLKCHHLKGNQLAEYDPV---------KLTLQHVNSNQLLDKA----TEEDSQVPSRR-DTNGS : 151
T2lektdom : -PGSLIKLQGCREND--SRQKAEQIEG---------NSKLRHVGSNLLLDSR-----TAKSGGLSTE-VTGP- : 150
T3lektdom : -VQLKACTYKGHKTVVTGEQIAEIQK----------DQLLYNPFLKMTLS--------ANGEHPSTV-SLNPS : 155
T4lektdom : -NYVGMQNCPKDGFPVPANIIAHFKE---------DGTLFHPHSGLCLS--AYRTPEGRPDV-QQR-TTDAL : 164
T5lektdom : -----GAVRLHPCDNRNKGLKALHKSTSVFHPELVNHIVFENNQQLLCLEG------NFSQKILKLA-AIDPV : 154
T6lektdom : -LGLGSCHFTGKNSQVPKDEDAELAQ---------DQLLRNSGSGTTLT--------SQDKKPAQA-PLNPS : 156
T7lektdom : ------SKVMITHCNLNEFKEAQYFKNL---------HRFTHIPSGKCLDR------SEVLHQVFTS-NLDSS : 152
T8lektdom : EKPTLEPCSKAAKN--RLHIYADFKP---------GGAVINRDLKRTL---EMKKDLLGSHVLVSQ-TTST- : 161
T9lektdom : RMPTLKRCEDVAR---PTQRLADFTQ---------SGPDVSRAIGRCLEV-EMSKDANFGLRLVSQ-RTSG- : 163
T10lektdom : SPVTLYDCHS-----MKGNQLAKYRKD---------KTLYHPVSGSTFDC------SESDHRIFVN-TNPS : 165
T11lektdom : ---SSDPPRLMKCHGSGGSQQATFGK---------NNRIYQVSVGQCLRAVDP---LGQKGSVAQA-IIDGS : 172
T12lektdom : DTLIMHLCEET----APENQKEILQE---------DGSLFHEQSKKTLQ--AARKESSDSFVPLAR-DTTNS : 161
T13lektdom : ---VILQNCTEEG-LAIHQQHADFQE---------NGMIVHILSGKCFE--AVVQENNKDLY--AR-PADGK : 158
T14lektdom : -----GPVIMLKCHHMRGNQLAEYDAE---------RLTLRHVNSNQLLDEP----SEEDKMVPTQQ-DTSGS : 151
T15lektdom : ------------------QQLTKT----------GSHIEHIASHLLIDTDMFGDGTENGKEIGAN-PTESS : 160
T16lektdom : MSSPGSPVILQMCNPREGKQKARRK----------GSPLQHSVSGLCLET---------KPAQLATSKLQAD : 151

QXW
T1lektdom : R-SLQQLLRNVTLPEIF------ : 167
T2lektdom : ALSLQQKFTLNLQQ--------- : 164
T3lektdom : DPLLKQILSQND---------- : 167
T4lektdom : DKNLISFEK------------- : 174
T5lektdom : KPYLKQKFEKYYEA--------- : 168
T6lektdom : DPHLLLFV-------------- : 165
T7lektdom : KTTLKQEMNNIHSV--------- : 166
T8lektdom : ---LVQEIQHTVRDWGQTNSQ-- : 179
T9lektdom : ---LKQMIRNWIKHARH------ : 177
T10lektdom : SLTLQQLFEHTNSTVLEKFNRN- : 187
T11lektdom : S-SLQQHLEG------------- : 181
T12lektdom : D-HLKQFFKERML---------- : 173
T13lektdom : A-RLQQRFDQINAVDER------ : 174
T14lektdom : R-SLQQLLRNMTLGT-------- : 165
T15lektdom : LMSLHQDMVSS------------ : 171
T16lektdom : AQALQQQLLPHT---------- : 163

FIG. 2

MUC1 full coding and secreted constructs

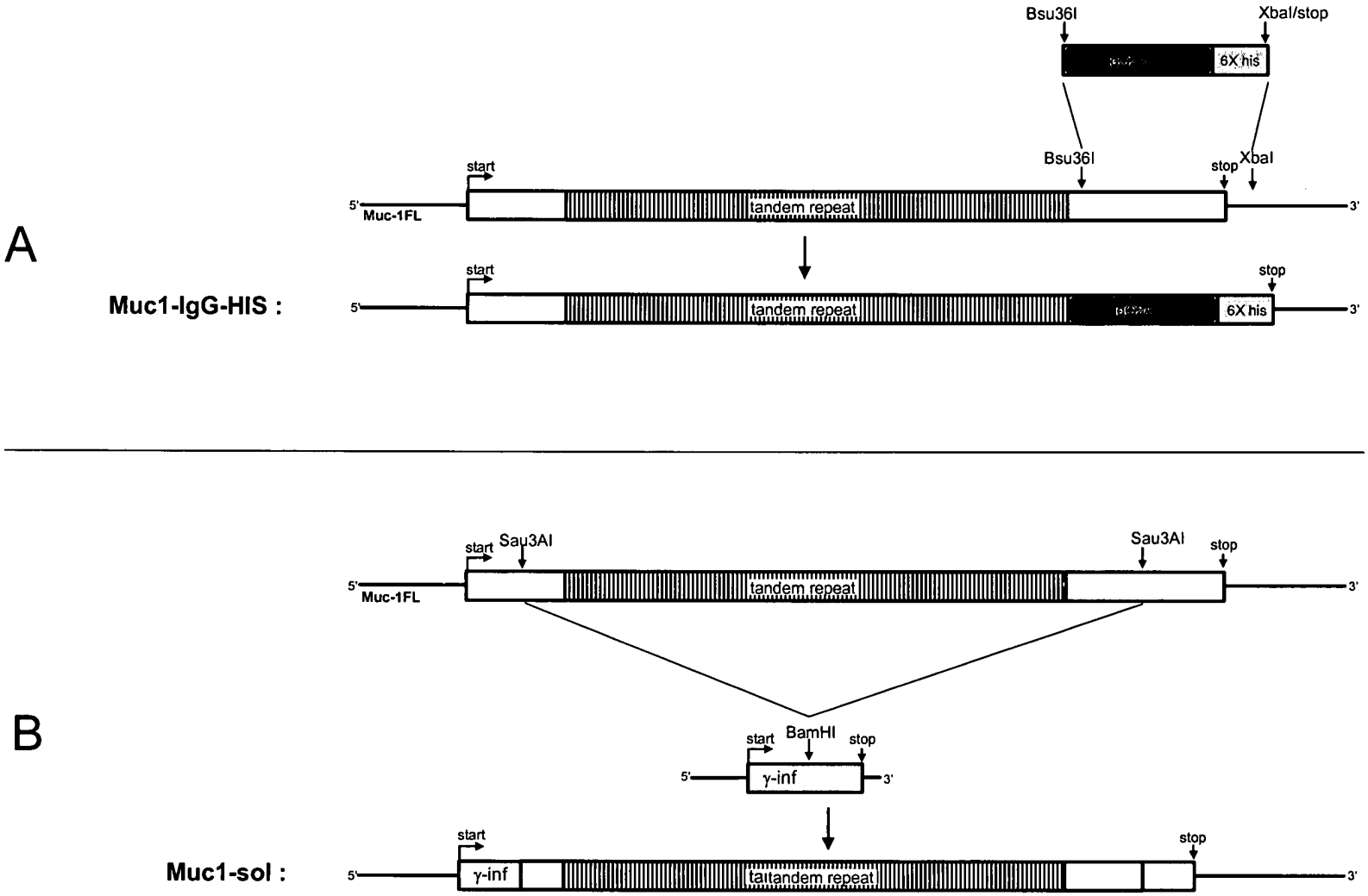
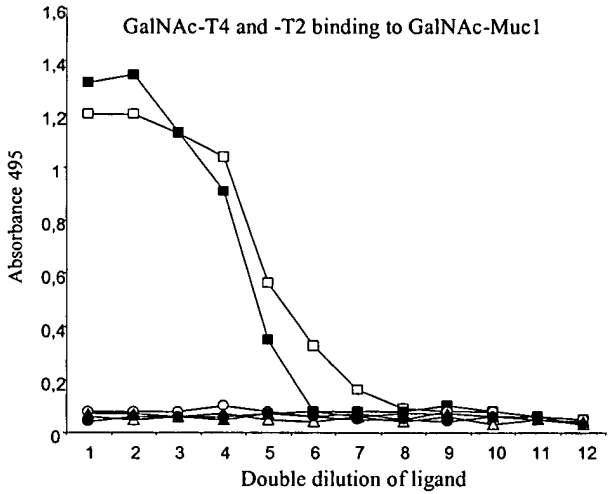


FIG. 3

A.


GalNAc-T4 and -T2 binding to GalNAc-Muc1
Absorbance 495
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
1 2 3 4 5 6 7 8 9 10 11 12
Double dilution of ligand

B.

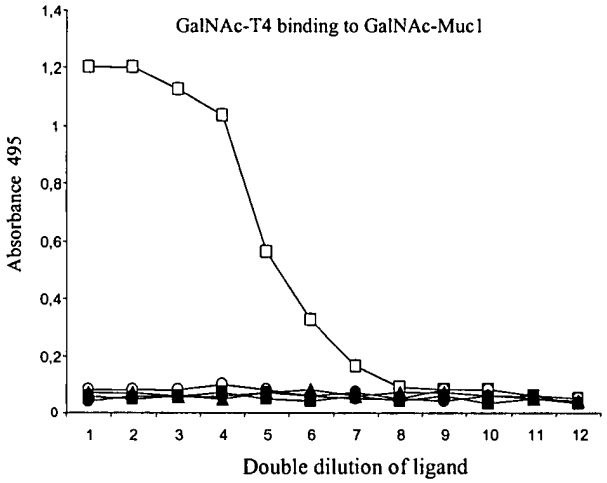

GalNAc-T4 binding to GalNAc-Muc1
Absorbance 495
1,4
1,2
1
0,8
0,6
0,4
0,2
0
1 2 3 4 5 6 7 8 9 10 11 12
Double dilution of ligand

FIG. 4

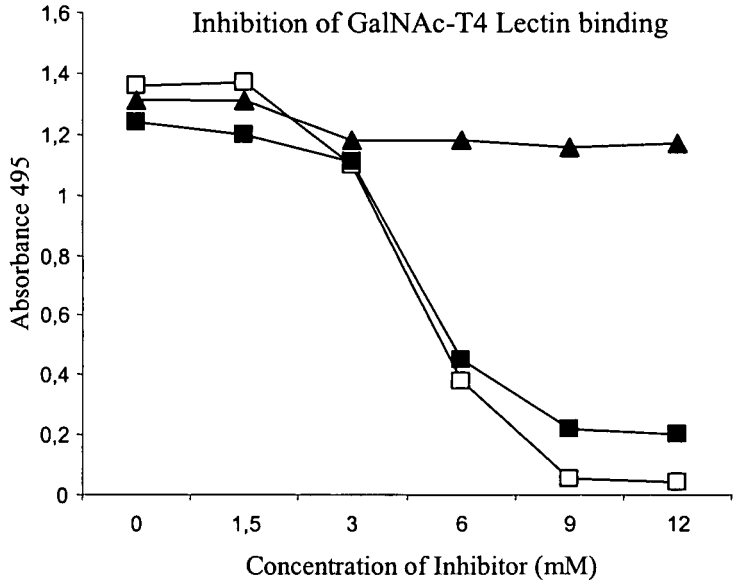

Inhibition of GalNAc-T4 Lectin binding
Absorbance 495
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
0
1,5
3
6
9
12
Concentration of Inhibitor (mM)

FIG. 5

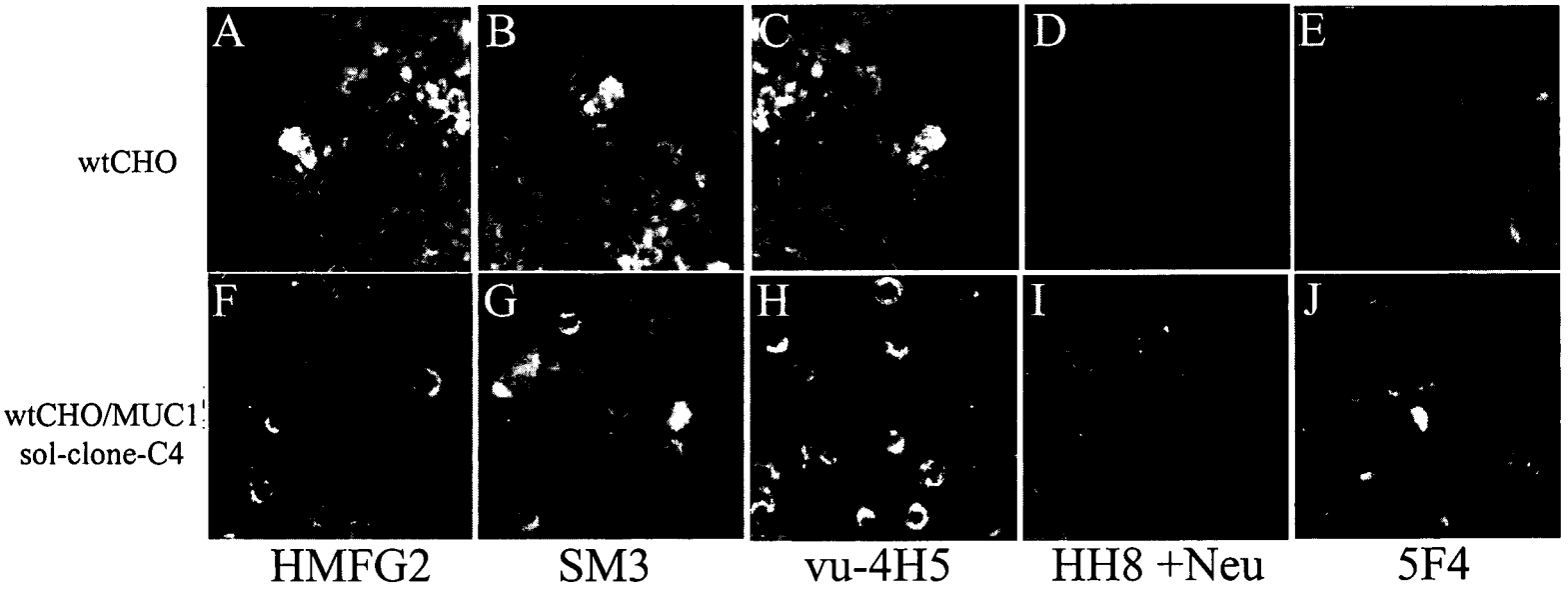

A
B
C
D
E
wtCHO
F
G
H
I
J
wtCHO/MUC1
sol-clone-C4
HMFG2
SM3
vu-4H5
HH8 +Neu
5F4

FIG. 6

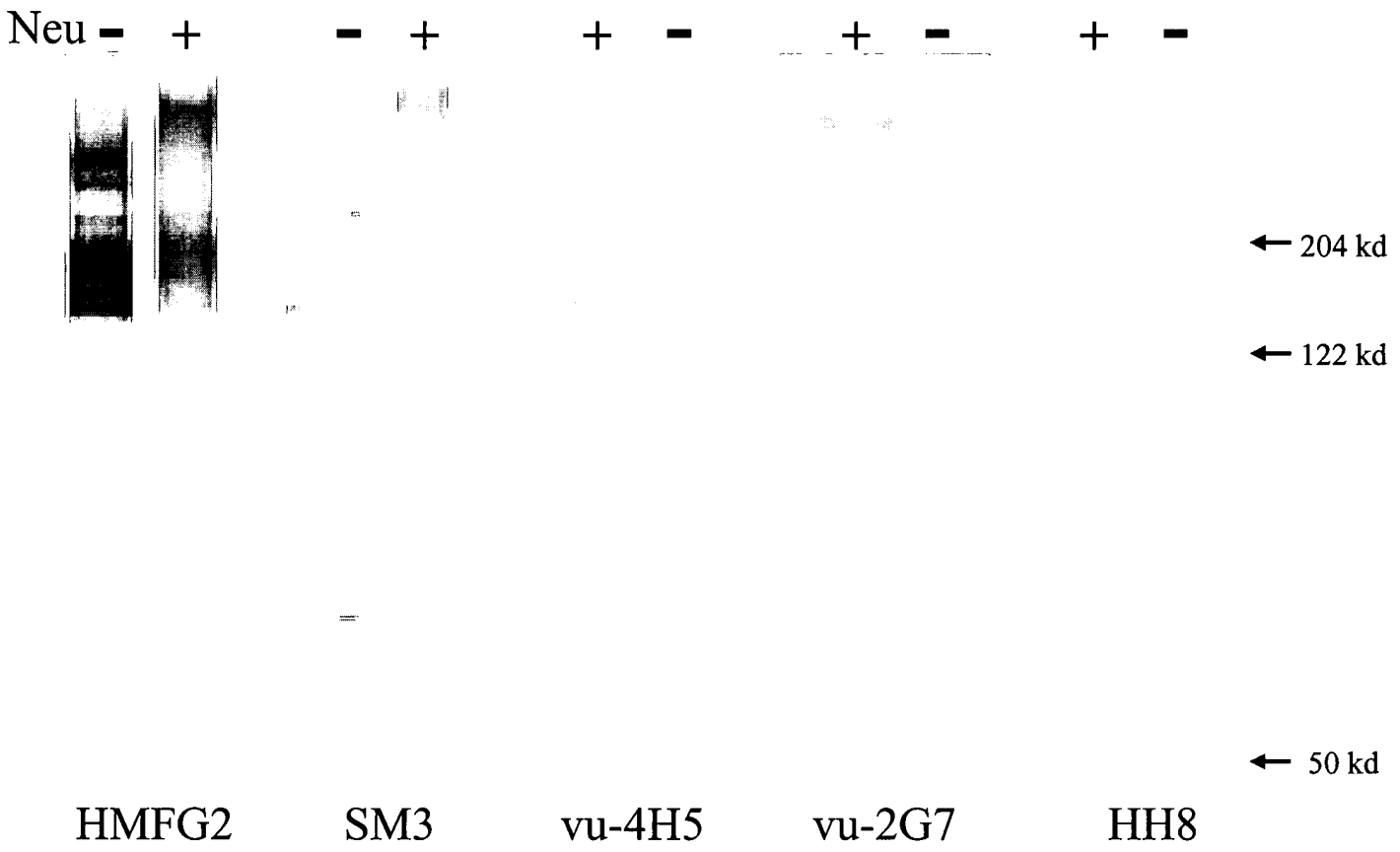


FIG. 7

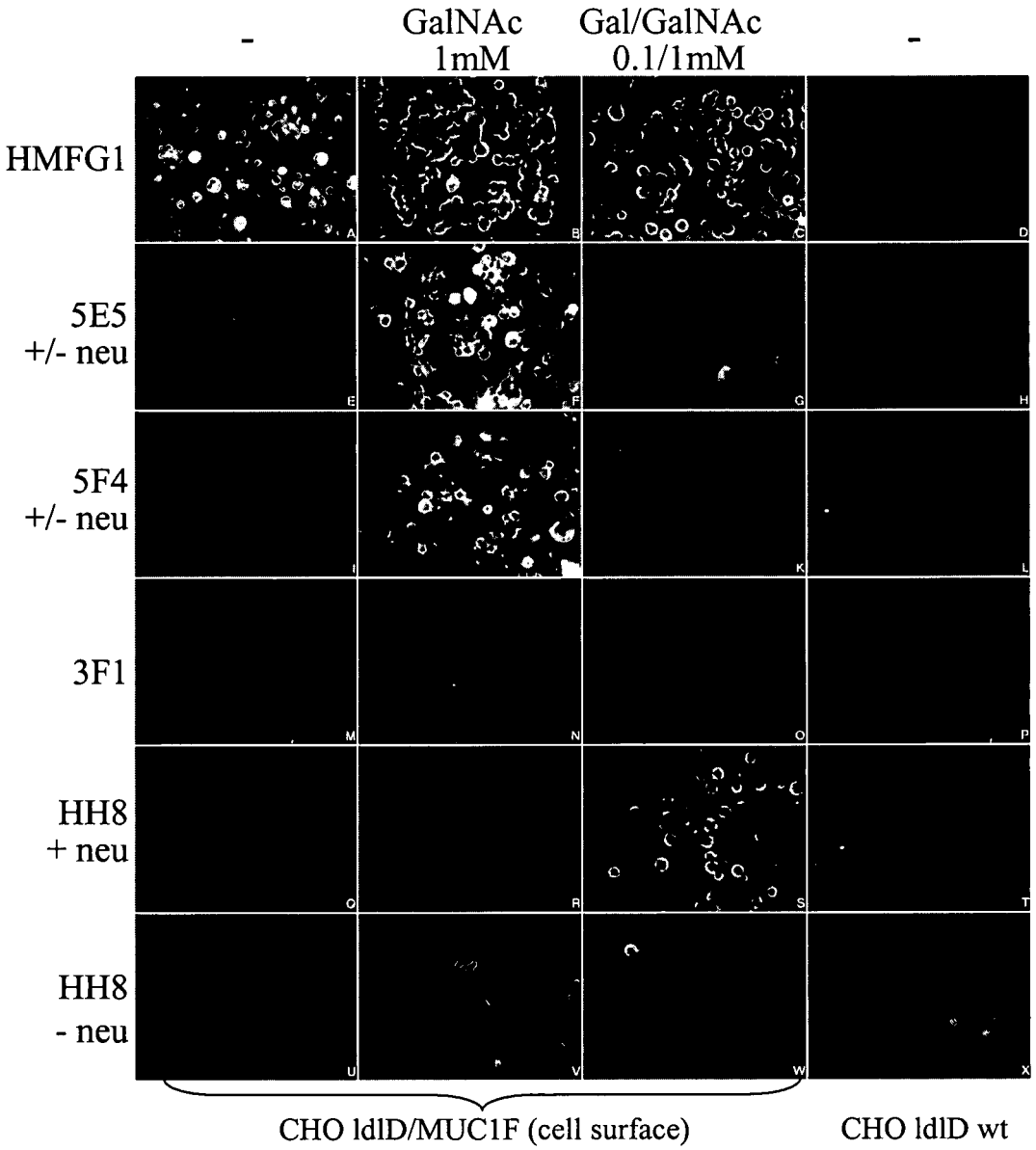

-
GalNAc
1mM
Gal/GalNAc
0.1/1mM
-
HMFG1
5E5
+/- neu
5F4
+/- neu
3F1
HH8
+ neu
HH8
- neu
A
B
C
D
E
F
G
H
I
K
L
M
N
O
P
Q
R
S
T
U
V
W
X
CHO ldlD/MUC1F (cell surface)
CHO ldlD wt

FIG. 8

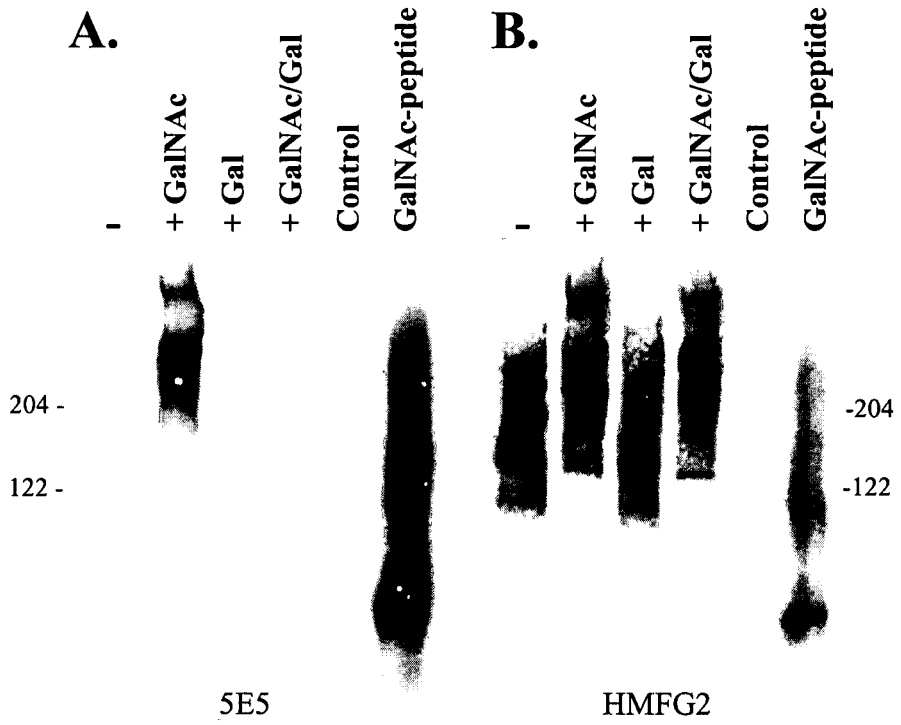

A.
B.
-
+ GalNAc
+ Gal
+ GalNAc/Gal
Control
GalNAc-peptide
204 -
122 -
-204
-122
5E5
HMFG2

FIG. 9

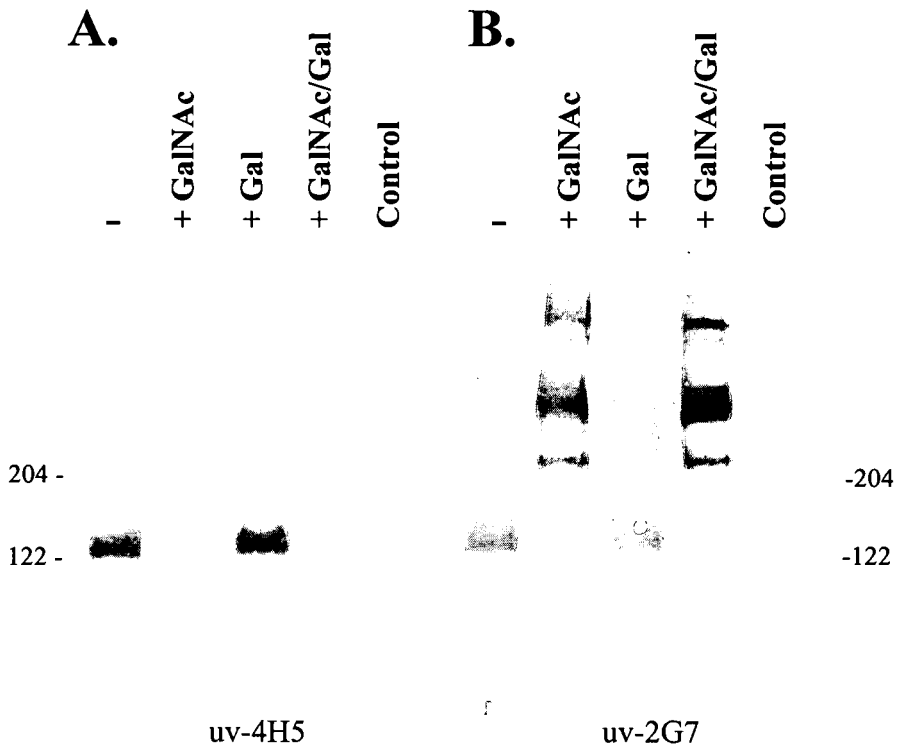


FIG. 10

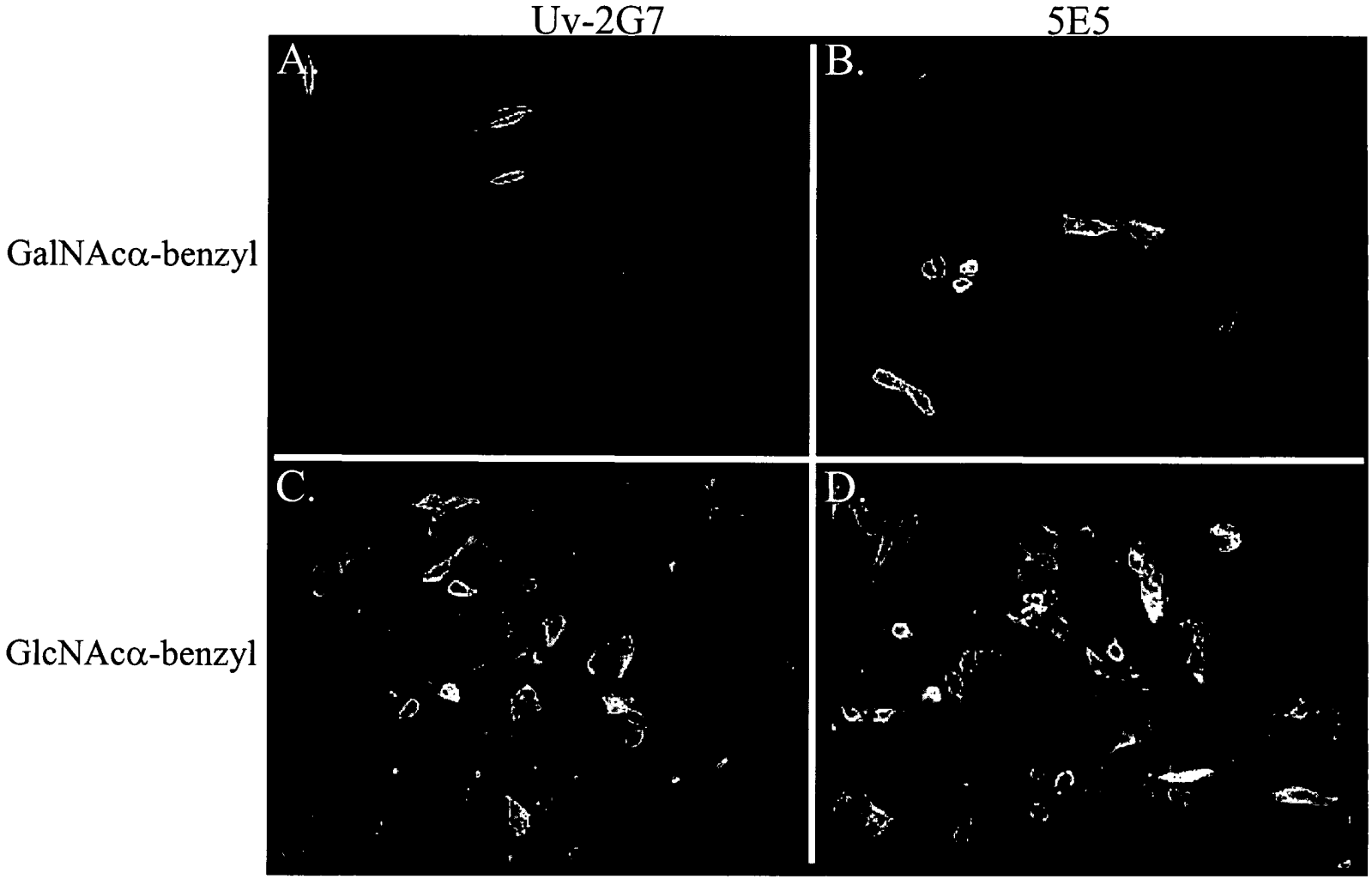


FIG. 11

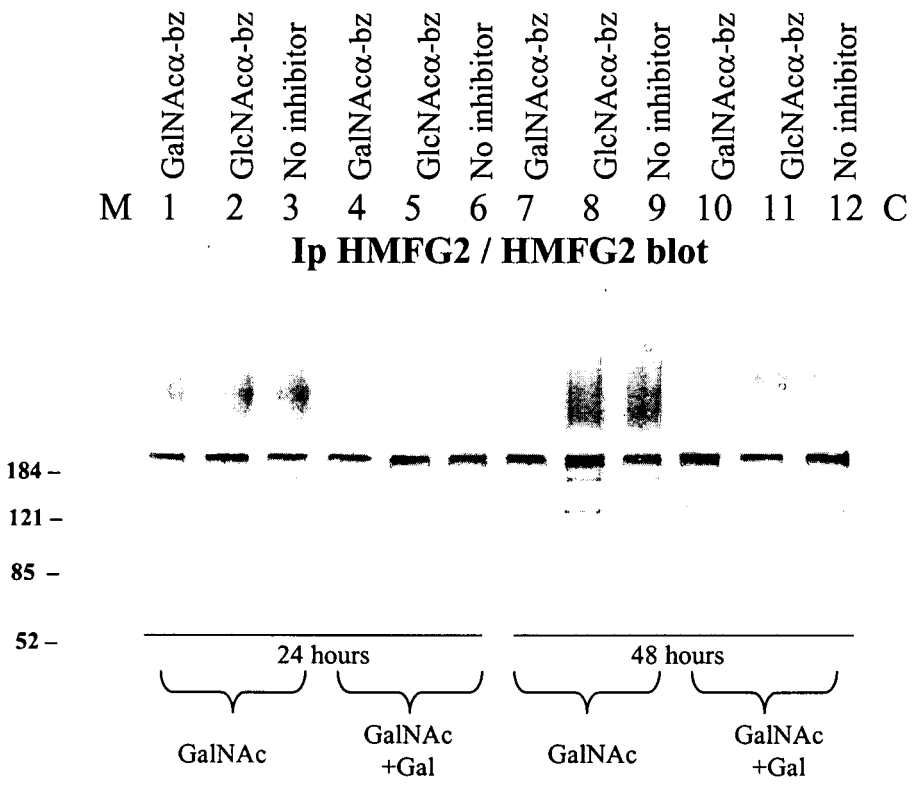

GalNAcα-bz
GlcNAcα-bz
No inhibitor
GalNAcα-bz
GlcNAcα-bz
No inhibitor
GalNAcα-bz
GlcNAcα-bz
No inhibitor
GalNAcα-bz
GlcNAcα-bz
No inhibitor
M 1 2 3 4 5 6 7 8 9 10 11 12 C
Ip HMFG2 / HMFG2 blot
184 –
121 –
85 –
52 –
24 hours
48 hours
GalNAc
GalNAc +Gal
GalNAc
GalNAc +Gal

FIG. 12

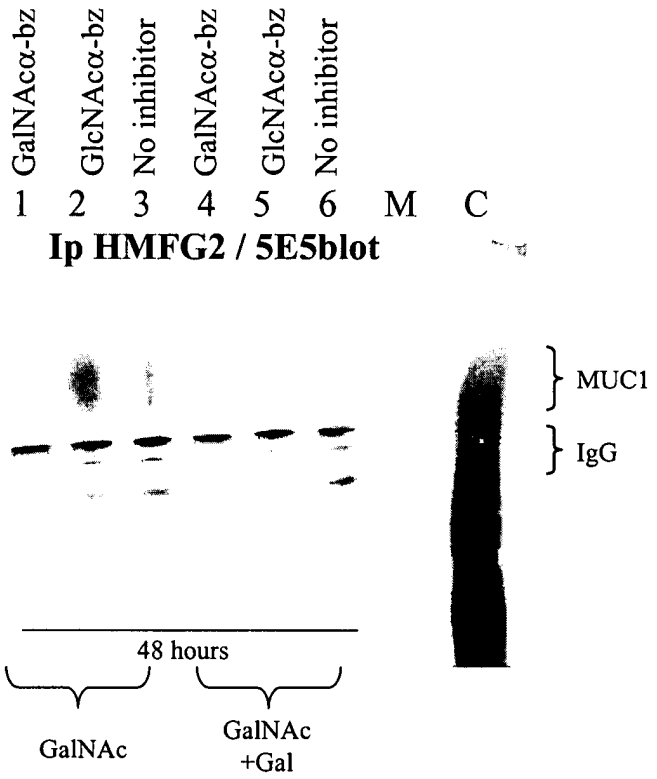

GalNAcα-bz
GlcNAcα-bz
No inhibitor
GalNAcα-bz
GlcNAcα-bz
No inhibitor
1 2 3 4 5 6 M C
Ip HMFG2 / 5E5blot
MUC1
IgG
48 hours
GalNAc
GalNAc
+Gal

FIG. 13

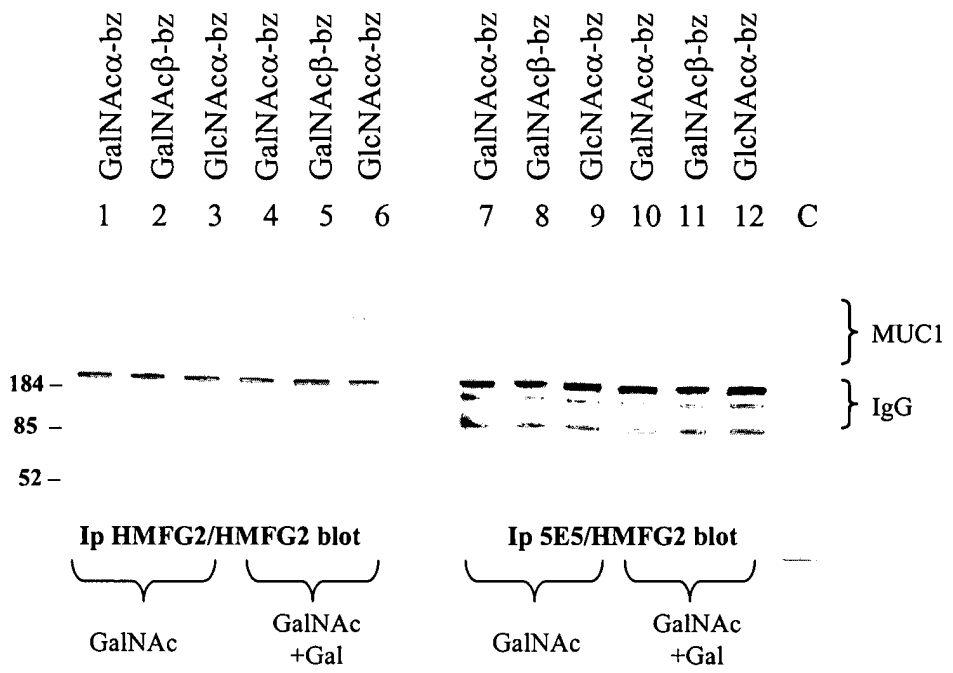

GalNAcα-bz
GalNAcβ-bz
GlcNAcα-bz
GalNAcα-bz
GalNAcβ-bz
GlcNAcα-bz
GalNAcα-bz
GalNAcβ-bz
GlcNAcα-bz
GalNAcα-bz
GalNAcβ-bz
GlcNAcα-bz
1 2 3 4 5 6 7 8 9 10 11 12 C
MUC1
IgG
184 –
85 –
52 –
Ip HMFG2/HMFG2 blot
Ip 5E5/HMFG2 blot
GalNAc
GalNAc +Gal
GalNAc
GalNAc +Gal

FIG. 14

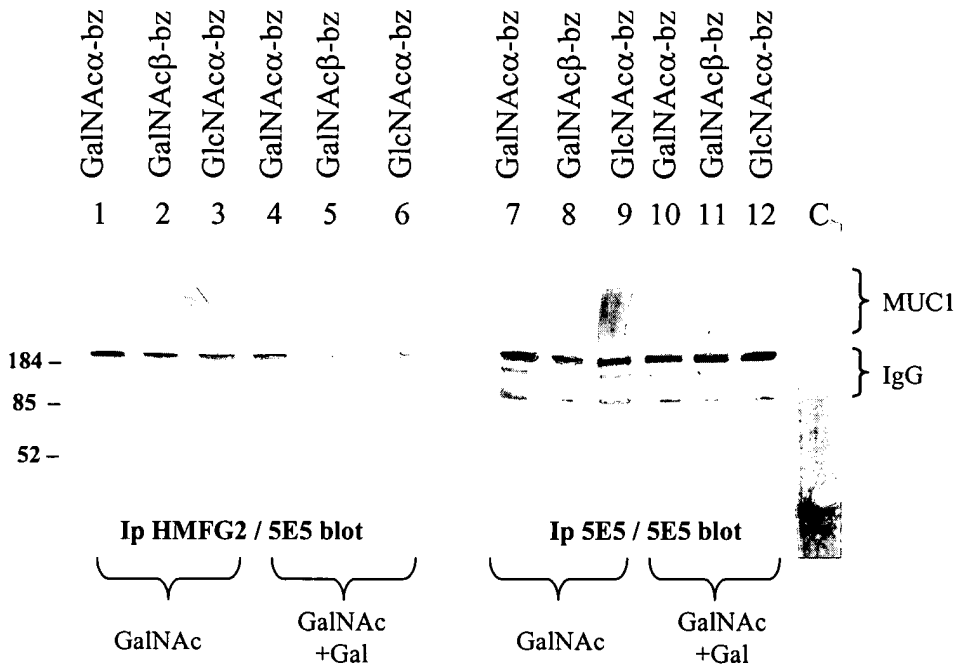

GalNAcα-bz
GalNAcβ-bz
GlcNAcα-bz
GalNAcα-bz
GalNAcβ-bz
GlcNAcα-bz
GalNAcα-bz
GalNAcβ-bz
GlcNAcα-bz
GalNAcα-bz
GalNAcβ-bz
GlcNAcα-bz
1 2 3 4 5 6 7 8 9 10 11 12 C
MUC1
IgG
184 –
85 –
52 –
Ip HMFG2 / 5E5 blot
Ip 5E5 / 5E5 blot
GalNAc
GalNAc +Gal
GalNAc
GalNAc +Gal

FIG. 15

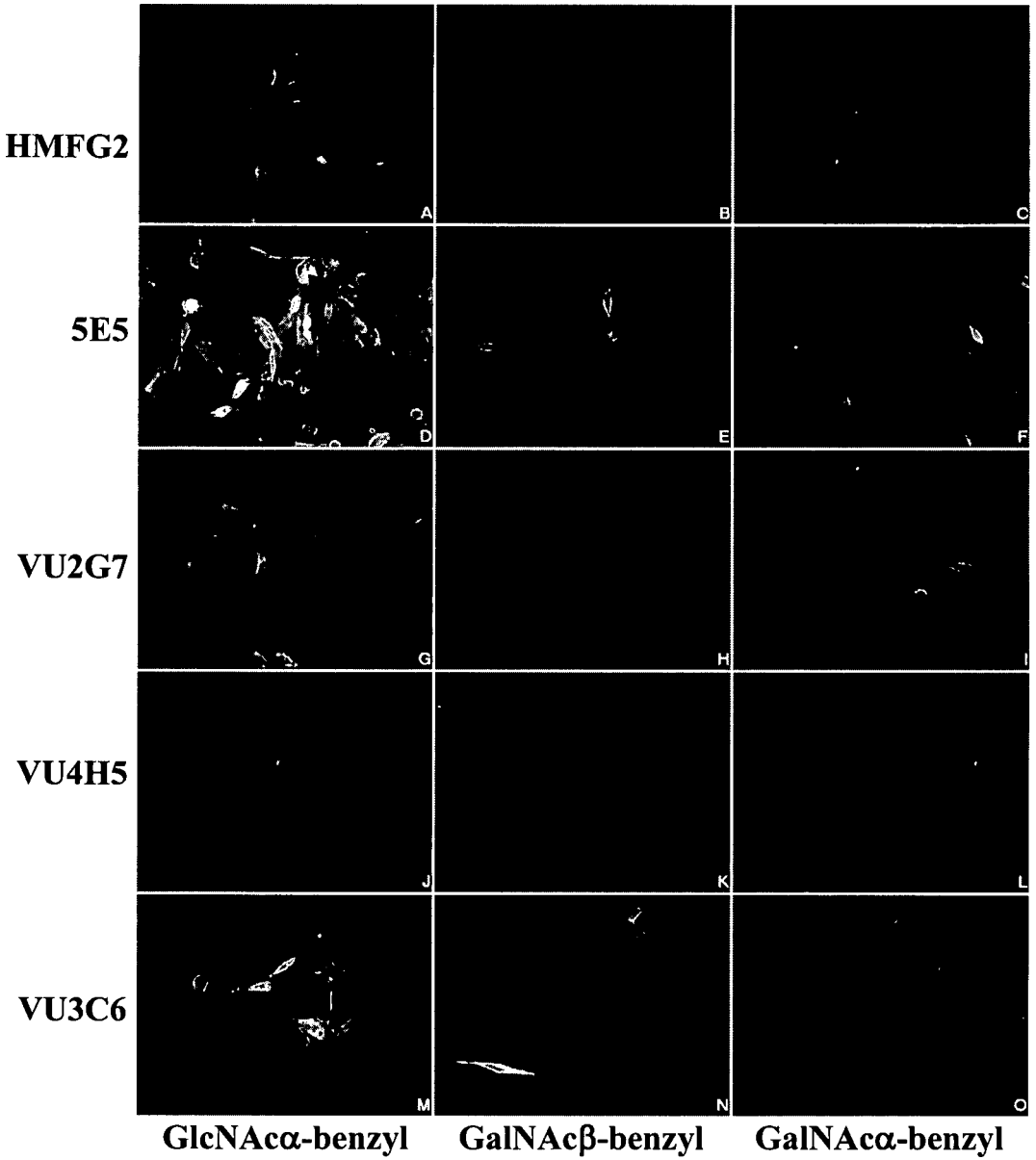

HMFG2
5E5
VU2G7
VU4H5
VU3C6
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
GlcNAcα-benzyl
GalNAcβ-benzyl
GalNAcα-benzyl

FIG. 16

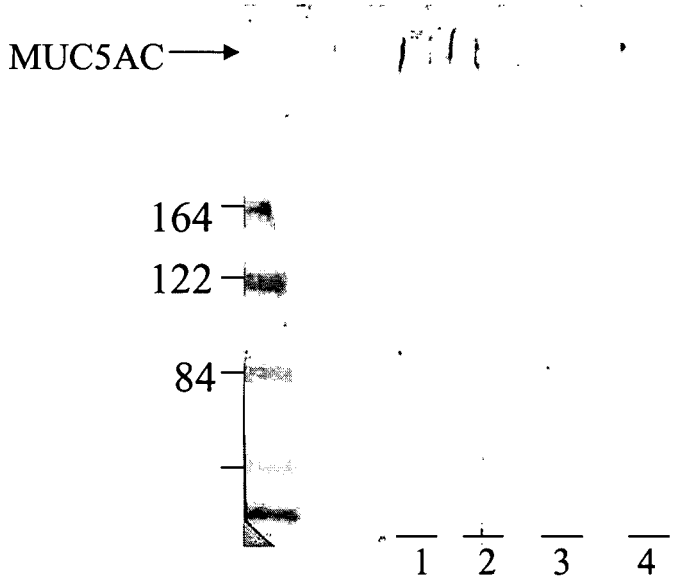


FIG. 17

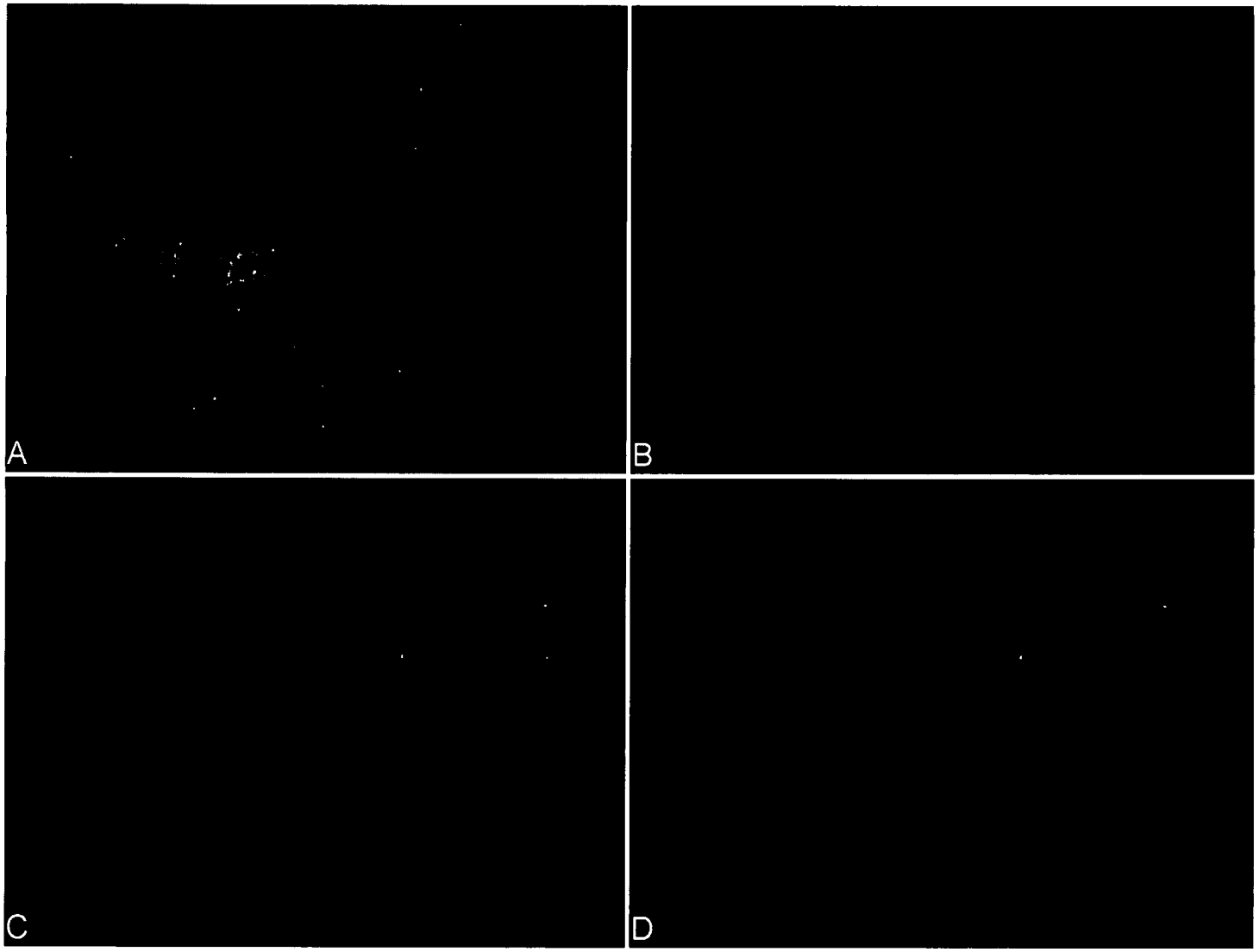


FIG. 18